# Ionic liquid-mediated recovery of carotenoids from the *Bactris gasipaes* fruit waste and their application in food-packaging chitosan films

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**Abstract:** In this work, the extraction of carotenoids from the fruit of the Amazonian *Bactris gasipaes* was developed. Ethanolic and aqueous solutions of ionic liquids (ILs), and surfactants were evaluated on the extraction of these pigments. Thus, we developed an optimized sustainable downstream process mediated by the best solvent with further isolation of the carotenoids and the recyclability of the IL used. The process was characterized, not only in terms of efficiency but also regarding its environmental impact. The recyclability of the solvents, as well as the high efficiency (maximum yield of extraction of carotenoids = 88.7 ± 0.9 $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$) and the low environmental impact of the integrated process developed in this work, were demonstrated. In the end, in order to incoporate functional activity for an alternative food-packaging material, carotenoids were were sucessufly applied on the preparation of chitosan-based films with excellent resulsts regarding their mechanical parameters and antioxidant activity.

**Keyworks:** all-*trans*-β-carotene; sustainable downstream process; ionic liquid; bio-based material; sustainable material;

## Introduction

The exploration of natural resources to produce consumer goods and commodities is being deeply investigated. New or improved production technologies have been developed to mitigate the residues disposal, while simultaneously enhancing the valorization of by-products.[1] There is nowadays a real demand for the creation of more sustainable and efficient downstream processes able to recover bio compounds usually discarded in biological waste, in spite of their potential for a large range of commercial applications.[2,3] Food waste is, in this scenario, one of the major problems worldwide. Actually, the European commission is calling the attention for their impacts in a daily-base. It is estimated that in EU, around 88 million tones of food wastes are generated annually with associated costs around the 143 billion euros.[4] As it has been successively explained by authorities worldwide, wasting food has serious ethic and economic impacts. While the demand for food increases proportionally with the human population, being estimated to reach 9.1 billion by 2050,[5] the environmental impact of the residues generated is growing, severely damaging the environment and negatively impacting the climate (food alone is responsible for about 8% of Global Gas Emissions).[6] While reducing the food losses and wastes will help achieving the Sustainable Development Goals, the design of more sustainable downstream processes will allow the wastes industrial valorization, meeting the demands of both Circular Economy and Biorefinery strategies.[7–9]

In addition to Europe, for many developing countries, such as Brazil, India and China, with high social heterogeneity and cyclical economic crises, the reduction of food wastes is a huge challenge.[10] In Brazil, 41 million tons of food are wasted every year.[10] In contrast, Brazil has a massive food biodiversity, with 18% of the plants of the world,[11] and therefore, many unused raw materials to be explored, contributing thus for the

creation of new bio-based products.[12] In this context, the business of palm heart (*Bactris gasipaes*) is a very good example of an important agro-food activity from the Brazilian Amazonian region, originally cultivated by indigenous populations.[13] Palm hearts, known in Brazil as "palmito", are the edible stipe-apical meristem of the palm tree, the main agro-product of this species. Known as a predatory crop, the Brazilian Ministry of Agriculture (EMBRAPA), recommends that the palmito be harvested after the fruit ripening.[14] However, despite this recommendation, their fruits are usually discarded.[15] *Bactris gasipaes* fruits, popularly known as peach palm, are an excellent source of carotenoids and other secondary metabolites.[16] Carotenoids are natural pigments with high antioxidant and anti-inflammatory activity, and therefore, with commercial interest.[17] Depending of the application, the choice of the carotenoid method-extraction from food matrices is crucial to ensure safety for the end product.[18] Nevertheless, the most common and efficient industrial downstream processes to produce carotenoids are usually mediated by organic synthesis; or in some cases (lycopene and astaxanthin) by extraction mediated by conventional organic solvents. Despite the different natural sources available rich in carotenoids, their recovery from the biomass is not easy.[19] We have reviewed the different strategies employed on the recovery and purification of natural products, which included the use of membranes, imprinted polymers, chromatography and alternative solvents, including ionic liquids and deep eutectic solvents.[20,21] Synthetic dyes, which are considered as more stable and cheaper than their natural counterparts are usually reported as carcinogenic and allergenic for humans.[22] Therefore, strategies for obtaining natural, thermally stable and low toxic carotenoids are in high demanded, moreover when considering the rapid growth of the market of anti-oxidant products and formulations.[23–25]

Bio-based materials with antioxidant properties are nowadays one of the hottest topics in the food sector.[26] Non-ecofriendly packages have been considered as one of the most important environmental problems to be solved, mainly in what concerns the use of plastic.[27] Thinking on a Circular Economy perspective, chitosan-based films are good candidates to replace the widespread use of non-degradable materials. According to Guillard et al.[28] the replacement of 50% of plastic food packaging by alternative materials can generate savings of 56 million tons of plastic/year. Chitosan is the second most abundant polysaccharide in nature, being renewable, easy to obtain, eco-friendly and nontoxic.[27] Despite the chitosan-based films poor mechanical properties,[29] they are an excellent basis to develop packaging materials with antioxidant and antimicrobial activities that would improve the food shelf-life and the preservation of its organoleptic properties.[30,31]

This work addresses the development of the integrated process for the recovery of natural carotenoids from *Bactris gasipaes* fruits, namely the all-*trans*-β-carotene, all-*trans*-lycopene and the all-*trans*-γ-carotene.[16,32] Various ethanolic and aqueous solutions of ionic liquids (ILs), with and without tensioactive nature, and two common surfactants were evaluated. After selection of the best solvent to extract the carotenoids, the process conditions of the solid-liquid extraction, including the solid–liquid ratio $R_{(S/L)}$, the time of extraction, and the concentration of IL ($Conc_{IL}$), were studied and optimized. The carotenoids isolation was studied by applying water as anti-solvent. The environmental impact of the proposed process was evaluated through the analysis of carbon footprint and complete E-factor. In the end, the carotenoids purified and isolated were utilized on the preparation of chitosan-based films. The mechanical properties of the membranes prepared were tested, namely the tensile strength, Young's modulus, elongation at break,

thickness and elasticity. Moreover, the wettability and solubility of the membranes in water were also studied.

## Material and methods

**Fruits and raw materials.** The *Bactris gasipaes* fruits studied were collected in the Bahia State, northeast region of Brazil (Ilhéus city: 14º50'00.47''S, 39º01'51.98''W). The fruits belong to the same batch previously studied,[33] and their respective samples were pretreated as follows. Briefly, after manual seed removal, the peach palm edible parts were immediately frozen at -100 °C, lyophilized for 48 h and then stored at – 40°C. The biomass composition was performed following the proximal characterization method according to Association of Official Analytical Chemists (AOAC). [34]

**Chemicals.** 1-hexyl-3-methylimidazolium chloride ([$C_6$mim]Cl), 1-octyl-3-methylimidazolium chloride ([$C_8$mim]Cl), 1-decyl-3-methylimidazolium chloride ([$C_{10}$mim]Cl), 1-dodecyl-3-methylimidazolium chloride ([$C_{12}$mim]Cl), 1-tetradecyl-3-methylimidazolium chloride ([$C_{14}$mim]Cl), 1-butyl-3-methylimidazolium tetrafluoroborate ([$C_4$mim][$BF_4$]) were purchased from IOLITEC, with purities > 98%. 1-dodecyl-trimethylammonium bromide ([$N_{1,1,1,12}$]Br) and 1-tetradecyl-trimethylammonium bromide ([$N_{1,1,1,14}$]Br) were purchased from Alfa Aesar, with purities higher than 98%. 1-decyl-trimethylammonium bromide ([$N_{1,1,1,10}$]Br) and 1-decyl-trimethylammonium chloride ([$N_{1,1,1,10}$]Cl) (> 98% of purity) were purchased from TCI. SDS and Tween 20 were purchased from PanReac AppliChem, with purities > 98%. To prepare the bio-based films, chitosan from shrimp of medium molecular weight with a degree of deacetylation of 85% and glycerol were supplied by Sigma-Aldrich (St Louis, MO, USA). The antioxidant activity was performed using anhydrous sodium carbonate

(99%) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic) (ABTS, 99%) were purchased from Fluka (St Louis, MO). All other reagents used were of analytical grade.

**Solid-liquid extraction using organic solvents.** A conventional approach with pure acetone and ethanol, applied as controls, was performed to compare the yield of carotenoids extraction with the alternative extraction mediated by ILs and two traditional surfactants. The peach palm fruits were incubated for 90 min with a solid-liquid ratio of 0.1, meaning 0.5 g of biomass in 5 mL of solvent, at room temperature with homogenization at 80 RPM. After, the solutions were centrifuged (5000 RPM, 4 °C, 30 min) and the supernatant was stored at – 40°C for further analysis. The extracts were performed in triplicated being presented for discussion the average and respective standard deviations. The purification of the carotenoids obtained by these processes were mediated also by organic solvents, namely diethyl and petroleum ether. [16,33]

**Alternative solid-liquid extraction using ILs and common surfactants.** The potential of extraction of different ethanolic and aqueous solutions of surface-active ILs and common surfactants were evaluated regarding the recovery of carotenoids from the dried biomass. Included in the set of solvents tested are aqueous and ethanolic solutions of ILs from the imidazolium, ammonium and phosphonium families, polysorbate 20 (Tween 20) and sodium lauryl sulfate (SDS). For the solvents' screening, the concentration of 250 mM, $R_{(S/L)}$ of 0.1 and 90 min of time of exposure, were the conditions fixed. The samples were centrifuged at 80 RPM. The yield of extraction of carotenoids ($\mu g_{carotenoids} . g_{dried\ biomass}^{-1}$) was determined by HPLC-DAD using the method described by de Souza Mesquita et al. (2019).[33] The assays were performed in triplicate for each system and each

condition tested. The results were thus expressed considering the mean associated to the respective standard deviations.

After the screening with the alternative solvents, the most promising system, meaning the one having the highest yield of extraction for carotenoids from the peach palm fruit, was selected. The optimization of the process was executed by applying a central composite rotatable design (CCRD; $2^3$ plus axial) with six replicates at the central point, totalizing 20 extractions. The independent variables optimized were the solvent concentration ($C_{IL}$), time of extraction (t) and solid-liquid ratio ($R_{(S/L)}$). The results obtained were statistically verified for a confidence level of 95%. The surface responses were plotted changing two variables within the experimental range. The dependent variable here evaluated was the total yield of extraction of carotenoids, considering the amount of all-*trans*-lycopene, all-*trans*-β-carotene, and the all-*trans*-γ-carotene extracted, since these are the most abundant in this biomass. After analysing the RSM results, the best conditions for the carotenoids' extraction for the best solvent were determined, and the model was validated in triplicate (predicted x experimentally determined). The Statistica 12.0 software was used to analyse the results allowing the response surfaces determination.

**Carotenoids polishing and recycling of the best solvent.** After the validation and respective optimization of the alternative process, the carotenoid's recovery was performed by using water as an anti-solvent, which consequently allowed the precipitation of carotenoids. Five conditions were tested considering the determination of the amount of water required, namely 2x, 3x, 5x, 10x and 50x more water added to the carotenoids-rich extract with an initial volume of 5 mL. After isolation of the main solvents, consecutive cycles of extraction of carotenoids from new biomass samples were

tested aiming at to evaluate the performance of the best solvent after being recycles. In this work, 3 new cycles were carried and its performance evaluated by the analysis of the extraction efficiency ($\mu g_{carotenoids}.g_{biomass}^{-1}$).

**IL quantification.** The IL quantification was performed by ionic chromatography (bromide detection). The analysis of the samples was carried on a Dionex 2000i/SP Ion Chromatograph with conductivity detector. The bromide was analysed on a AS4A SC (25 cm X 4mm I.D) with an AG4A-SC ground column 4 mm I.D. It was detected by a suppressed conductivity detector using an Anion Micro-Membrane AMMS-I with regenerant of 25 mN of sulfuric acid. The injection volume was 10 µL, and the flow rate was 2.0 $mL.min^{-1}$. The chromatograms were recorded on a Chromjet integrator from Dionex.

**Thermal gravimetric assay (TGA).** TGA assays (SETSYS Evolution 1750 analyser - Setaram Instrumentation) were performed. Briefly, each sample was heated at a constant rate of 10 °$C.min^{-1}$ from 20 to 540 °C under a nitrogen flow of 200 $cm^3.min^{-1}$.

**Monosaccharides analysis.** Neutral monosaccharides were determined as alditol acetates by gas chromatography.[35] Briefly, the samplew were hydrolyzed using a solution of sulfuric acid (2 M at 120 °C for 1 h). The monosaccharides were reduced with sodium borohydride and acetylated using acetic anhydride. 2-Deoxyglucose was used as internal standard. The alditol acetates were analyzed by gas chromatography (GC) in a 30 m capillary column DB-225 (J&W Scientific, Folsom, CA, USA), with an internal diameter of 0.25 mm and a film thickness of 0.15 µm. The GC was equipped with a flame ionization detector (GC-FID Clarus 400, Perkin Elmer, MA, USA) and the monosaccharides were identified by retention time comparison with external standards.

The analyses were performed in triplicate. The uronic acids were determined according to a modification of the 3-phenylphenol colorimetric method.[35] The samples (2 mg) were hydrolyzed using sulfuric acid (1 M at 100 °C for 1 h). A calibration curve was made with D-galacturonic acid (0-200 mg $mL^{-1}$). The analyses were performed in triplicate.

**Environmental evaluation by determination of carbon footprint and complete E-factor.** The carbon footprint consists in the sum of greenhouse gas (GHG) emissions expressed as carbon dioxide equivalent ($CO_2$ eq) from a life cycle perspective, while the complete E-factor assesses the efficiency of a process by measuring the total amount of waste generated during the process, including water, relative to each isolated product. In both cases, the stages of fruit preparation, extraction and polishing were assessed. The carbon footprint considers the GHG emissions from the production of all chemicals, water and electricity consumed during the three stages. All data required for the environmental evaluation regarding chemicals, water and electricity consumed were obtained experimentally (Table S1 from ESI) and the GHG emission factors were taken from from Ecoinvent database version 3.5 (Ecoinvent, 2018) (Table S2 from ESI).[36] To calculate the complete E-factor, data on the amounts of waste generated from the use of $[N_{1,1,1,10}]Br$, acetone, ethanol, water, petroleum ether and diethyl ether in the integrated system were obtained during the experiments. The analysis was performed considering 4 distinct scenarios, which are different from each other considering the amount of raw-materials as well as their energy dependence. The scenario 1 and 2 are the conditions mediated by IL-extraction, without and with recycling of the raw-materials, respectively. The scenario 3 and 4 are different from each other considering the conventional organic solvent used, respectively acetone and ethanol; in both, carotenoid isolation was performed using ether mixtures.[16,33] Further details on the calculation of carbon footprint and complete E-factor can be found in the ESI.

**Chitosan biofilms preparation.** The chitosan solutions were prepared by dissolving 1.5% (w:v) of chitosan in 5% (v:v) of acetic acid aqueous solution under stirring for 16h at room temperature. The films were divided into 3 samples. In each film sample, a different amount of carotenoids was added, namely 0.025% (w:w), 0.050% (w:w) and 0.100% (w:w), meaning the amount of carotenoids *per* total amount of chitosan. In the biofilms preparation, the carotenoids used were those obtained after purification with the best solvent and acetone. To prepare the chitosan-based films (n = 6), after the addition and homogenization of glycerol (0.75 %, w:w), the pure carotenoids in ethanol were added to the mixture and homogenized by ultra-dispersor at 19000 RPM for 3 min. After, the biofilms were filtrated, degassed and transferred to a pexiglass plate (144 $cm^2$ with 3 mm deep). The plates were placed in the oven for 16h at 35°C to form the film by solvent casting. Biofilms without carotenoids were done by the same procedure to be evaluated as controls.

**Chitosan biofilms characterization**

**Thickness and mechanical assays.** The thickness of the films produced was evaluated after the complete drying of the material, one day after their preparation. These measurements were performed using a hand-held digital micrometer (Mitutoyo Corporation). Six measurements were taken in random areas of the films. The results were expressed as the mean ± respective standard deviations and used for the calculation of the contact area ($mm^2$). The mechanical properties of the chitosan-based films, with and without carotenoids, were evaluated by uniaxial tensile tests at room temperature with monitored air humidity (model Ta.Hdi, Stable Micro Systems) equipped with fixed grips lined with thin rubber in the ends. The films were cut in strips with 90 mm length and 10 mm wide for the determination of their tensile properties. The terminal positions of the

269 films were fixed in the grips with an initial separation settled at 50 mm. The crosshead
270 speed was set at constant rate of 0.5 mm.s$^{-1}$. The contact area (mm²), Young's modulus
271 ($E$), tensile strength or stress at break ($\sigma_b$) and elongation or strain at break ($\varepsilon_b$) were
272 determined from stress-strain curves obtained from uniaxial tensile testes to film failure.
273 These parameters were calculated based on ASTM D 882-83 standard method. All the
274 analysis were performed using at least 6 replicates and adopting the methodologies
275 described elsewhere.[37]
276
277 **Water contact angle.** To estimate the wettability of the films, the contact angle of water
278 molecules on the films' surface was performed using a contact angle measuring system
279 (OCA 20, Data-physics) at room temperature and with air humidity control. 3 µL of
280 ultrapure water was dispensed as a drop in the surface of each film (1 x 10 cm) using a
281 microsyringe. The contact angles of the drops were calculated using an image obtained
282 by the software dataphysics SCA20_M4, using the Laplace-Young method. All the
283 analysis were performed at least 40 times in both sides of the chitosan-based films.
284
285 **Solubility.** The film solubility was determined placing one square (4 cm$^2$) of each film
286 prepared in 30 mL of 50:50 (V/V) water:ethanol mixture, at room temperature, with
287 orbital agitation (80 rpm) for a maximum of 7 days. Then, the films were placed in an
288 oven at 105 °C for 16 h. After cooling down to room temperature, the films were weighed.
289 The solubility was determined by the percentage of weight loss calculated as follows:
290 $$Weight\ loss\ (\%) = 100 \times \frac{m_b - m_a}{m_b} \quad (\textbf{\textit{Eq.1}})$$
291 where $m_b$ and $m_a$ are the weight of dry film before and after being immersed in the
292 water:ethanol mixture, respectively. This determination was performed in triplicate. The

films moisture was determined in triplicate by measuring their loss of weight, upon drying in an oven at 105 °C until reaching a constant weight (dry film weight).

**Antioxidant activity.** The antioxidant analysis of the films was evaluated by the method of 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS, described in literature.[38] The same approach was already used in chitosan-based films in previous works of the group.[37–39] Briefly, one square (1 $cm^2$) of each film was placed in 3 mL of $ABTS^{+\cdot}$ solution and after 15 min (T0) the absorbance of the solution at 734 nm (Jenway 6405 UV/Vis) was measured. The antioxidant activity of the films was monitored after 01, 05 and 20 days post preparation. All measurements were performed at least 4 times. The antioxidant activity was expressed by the percentage of inhibition of the $ABTS^{+\cdot}$, calculated by equation 1:

$$inhibition\ ratio\ (\%) = 100 \times \frac{A_b - A_f}{A_b} \quad (\textbf{\textit{Eq. 2}})$$

where $A_b$ and $A_f$ are the absorbance of the blank (without any film) and films' solutions, respectively.

## Results and discussion

**Optimization of IL type and solid-liquid extraction conditions.** Considering the high hydrophobicity of carotenoids and the purity levels required for their application on the preparation of biodegradable chitosan-based membranes, the first step carried on this work was the proper selection of the most efficient solvent to recover the carotenoids. In our previous work, recent published,[33] we developed a downstream process mediated by non-tensioactive imidazolium IL ($[C_4mim][BF_4]$), which was able to recovery a total of 143 $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$, also using peach palm fruits. However, some gaps considering the safety of applying these pigments is questionable, since IL-residual may be present into the carotenoid extract. Besides, the extracts were obtained using ultrasound-assisted as main homogenization technique, which increases the final cost and

difficult the scaling-up of the process. In this work, in order to facilitate and improve the extraction procedure, and envisioning an application under the food industry, we tested the extraction efficiency of eleven ILs (with and without tensioactive nature) and two common surfactants was tested in both aqueous and ethanolic solutions (Figure 1) were tested. The common tensioactive compounds, sodium lauryl sulfate (SDS, anionic) and polysorbate 20 (Tween 20, non-ionic), were selected since they were reported as efficient solvents on the recovery of carotenoids from other rich-carotenoids matrices.[40–43]

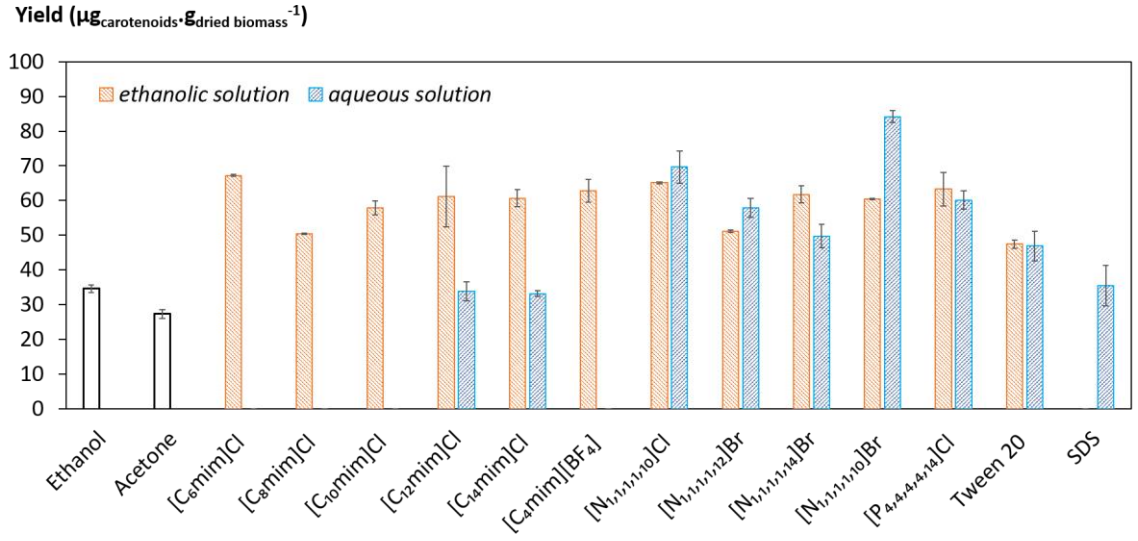



**Figure 1**. Screening of eleven ILs and two common surfactants on the extraction of carotenoids from peach palm fruits using aqueous (▨) and ethanolic (▨) solutions. Ethanol and acetone were used as controls (white bars).

The results depicted in Figure 1 suggest that, for the extraction of carotenoids, the use of aqueous solutions (blue bars) instead of ethanolic solutions (orange bars) is the best approach. The conventional extractions by acetone and ethanol achieved the lowest yields of extraction obtained in the present work; 35 ± 1 $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$ and 27 ± 1 $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$, respectively. Moreover, all aqueous solutions composed of ILs had yields of extraction equivalent or higher than those reported for conventional organic

solvents. Actually, the aqueous solutions of non-tensioactive ILs, namely 1-hexyl-3-methylimidazolium chloride [$C_6$mim]Cl, 1-octyl-3-methylimidazolium chloride [$C_8$mim]Cl, 1-decyl-3-methylimidazolium chloride [$C_{10}$mim]Cl, 1-Butyl-3-methylimidazolium tetrafluoroborate [$C_4$mim][$BF_4$], were not able to extract carotenoids using shaker homogenization,[41,44].

The poor capacity of non-tensioactive ILs to extract hydrophobic compounds has been discussed in other works and justified by the poor affinity on non-tensioactive solvents to interact with the membranes of the cells and by their lower capacity to solubilize hydrophobic compounds in water. A more aggressive homogenization technique seem to be necessary in order to extract lipophilic compounds using non-tensioactive IL, as showed in some recent works.[33,45,46] Moreover, our results also suggest that aqueous solutions of [$N_{1,1,1,10}$]Br are the best solvent to extract the carotenoids, achieving an extraction yield of 84 ± 2 $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$.

In an attempt to design a sustainable process, the aqueous solution of [$N_{1,1,1,10}$]Br, selected was further used on the optimization of the process conditions by applying a $2^3$ factorial planning. Here, the solid-liquid ratio [$R_{(S/L)}$], concentration of [$N_{1,1,1,10}$]Br ($Conc_{IL}$) and time of extraction (time) were the conditions optimized (Table S3 from ESI). The yield of extraction of carotenoids was used as the dependent variable in the definition of the predictive model represented by Equation 1. The yield of extraction of the carotenoids experimentally determined and predicted, as well as the statistical analyses performed, are shown in Table S3 in ESI. The model was adjusted with a confidence level of 95% and considered as a highly predictive model. Briefly, the results show that the $R_{(S/L)}$ and the IL concentration were the independent variables with higher influence on the extraction yield of carotenoids. A maximum extraction yield of 92 $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$ was obtained for the conditions $R_{(S/L)}$ = 0.15, $Conc_{IL}$ = 140 mM at 20 min (Assay

10). Furthermore, the results showed the negligible impact of time on the yield of extraction, demonstrated by the extraction yields that do not change between 8.2 and 31.8 minutes (Figure 2 and Figure S1 from ESI), which is an advantage from the process point of view. The model was further validated using the optimal conditions ($R_{(S/L)}$ = 0.15, $Conc_{IL}$ = 140 mM in aqueous solution for 8.2 minutes). Therefore, the average extraction yield obtained in the validation tests was 88.7 ± 0.9 $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$, which corresponds to a relative deviation of 1.5%, evidencing the high confidence and accuracy of the model. Besides, comparing the optimized results using aqueous-solution of $[N_{1,1,1,10}]Br$ and acetone (as main conventional organic solvent), we achieved an equivalent HPLC-DAD profile for both extracts, showing that the alternative process developed in this work did not impairs the extraction of any carotenoid commonly present in peach palm (Figure 3, Table S4 from ESI).

$$\textbf{\textit{total carotenoid content}}\ (\mu g.g-1) = -23.61 + 252.18(x1) + 0.90(x2) - 0.003(x2)^2 \quad (\textit{Eq. 3})$$

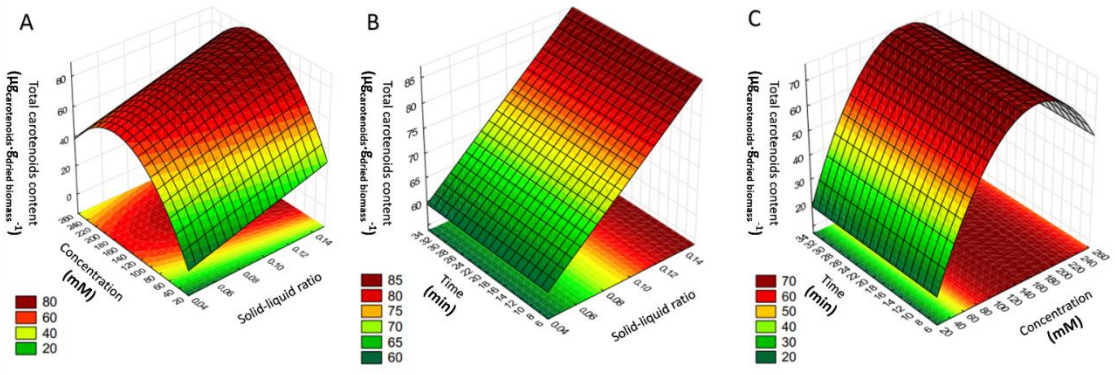



**Figure 2**. Response surface plots obtained for the factorial planning design ($2^3$) considering as independent variables the $R_{(S/L)}$, $Conc_{IL}$ (mM) and time (min) towards the yield of extraction of carotenoids (total carotenoids content) as the dependent variable.

The combination between independent variables were performed according to $R_{(S/L)}$ and $Conc_{IL}$ (**A**), Time and $R_{(S/L)}$ (**B**), $Conc_{IL}$ and Time (**C**).

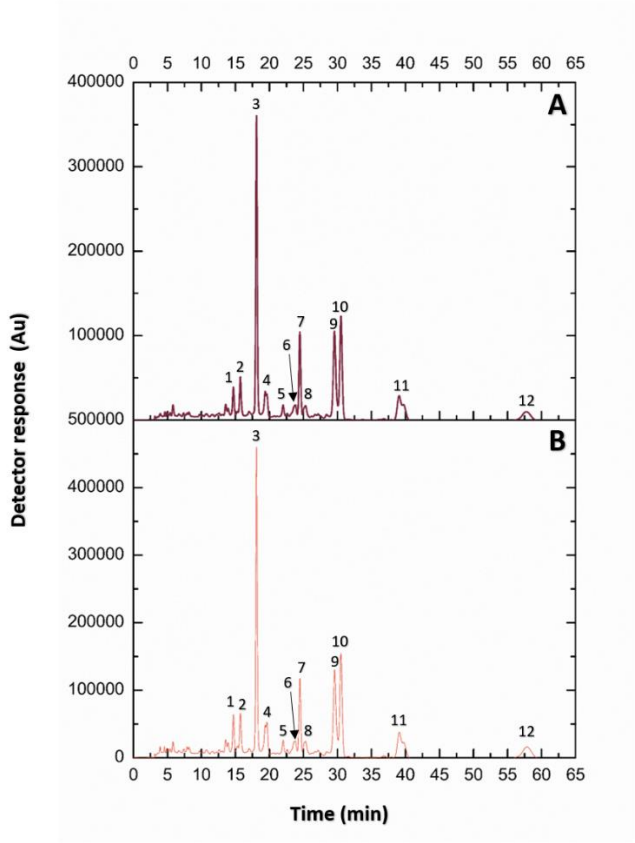



**Figure 3**. Chromatogram obtained by HPLC-DAD processed at 450 nm of the carotenoids extracts obtained by the conventional extraction using acetone (A) and by the alternative extraction performed by aqueous-solution of $[N_{1,1,1,10}]Br$. See chromatographic identification peaks in Table S4 from ESI.

**Carotenoids' polishing and IL' recycling.** To envision the industrial application of an extraction process, the solvents recyclability should be achieved. For this, two issues must be addressed, namely the complete isolation of the biomolecules from the solvents and the solvents recovery and reuse. In this work, considering the carotenoids hydrophobic nature and taking into account the structural features of $[N_{1,1,1,10}]Br$, the isolation of

carotenoids from the aqueous solution was investigated by applying water as an anti-solvent.[47] In this case, the rationale was that the addition of water is able to decrease significantly the IL concentration in solution, thus reducing the solvation potential of $[N_{1,1,1,10}]Br$,[48,49] decreasing the solubility of carotenoids in the medium. The amount of water was optimized by evaluating the effect of diluting the extract rich in carotenoids by a factor of 2, 3, 5, 10, and 50. As depicted in Figure S2 from ESI, the dilution conditions by factors of 2 and 3, were not able to isolate the carotenoids from the solvents. However, for the dilutions by factors of 5, 10 and 50, a second thin layer was formed, clearly evidencing the separation of carotenoids concentrated in the top phase and the aqueous solution of $[N_{1,1,1,10}]Br$ as the bottom phase (Figure S2C). Instead of the precipitation of carotenoids (Figure S2B), a pigmented top phase was formed (Figure S2C), a result already reported in some works using anti-solvents.[47,48,50] The "oily" layer formed, shows a high lipidic content of 15.6 ± 0.3 wt% (Table S5 from ESI). As shown in Figure S2, the carotenoids were suspended in the lipid's solution, which prevented them from being precipitated. According to literature,[32] the carotenoids from peach palm fruits are in intracellular lipid bodies, which explains the lipids' simultaneous extraction and presence in this second layer. From the 88.7 ± 0.7 $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$ extracted by using the aqueous solution of $[N_{1,1,1,10}]Br$, 99.9 ± 0.1 % of the total carotenoids were recovered in the lipidic phase formed, while no carotenoids were detected in the IL-rich phase. Besides the successful isolation of carotenoids, the recovery of the $[N_{1,1,1,10}]Br$ was also successfully achieved, with only 0.17 ± 0.02 % of IL in the carotenoids phase, while 93 ± 2 % could be recovered and reused in new cycles of extraction (the rest of the IL was lost in the biomass residues during extraction). Due to the economic interest in the extraction,purification, and processing of carotenoids,[51] we have designed an integrated process, reproducible and with scale-up viability, depicted in Figure 3. After the isolation

step, part of the water added was removed by evaporation and the aqueous solution of IL was reintroduced in a new cycle of (solid-liquid) extraction, to test the extractive viability of the solvent recycled. A total of 4 cycles (first extraction + 3 cycles of reutilization of the solvents) were carried as depicted in Figure S3 from ESI. The results show that in the first 2 cycles, the yields of extraction of all carotenoids (insets A, B, C and D of Figure S2 from ESI) were not compromised, however after the third cycle a decay on the extraction yield (ANOVA $p < 0.05$) was observed. Nevertheless, even with the decrease observed for the yield of extraction, after cycle 3, the results are still more than twice as higher as those obtained for ethanol and acetone (Figure S3 from ESI). To decrease even more the environmental impact of the final process, the water removed may be again used as anti-solvent as also depicted in Figure 4.

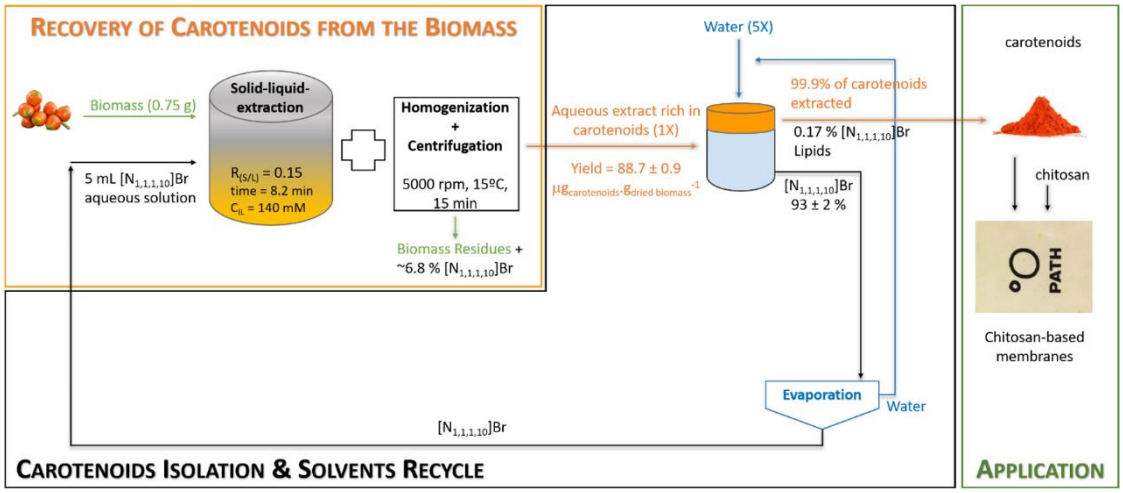



**Figure 4**. Schematic representation of the integrated process developed in this work contemplating the recovery of carotenoids from the biomass, followed by their isolation and solvents' recycle.

In addition to the recyclability potential of the IL, its selectivity on the extraction process was also experimentally assessed. According to Cláudio et al. (2014),[52] the structural integrity of a biomass sample after the extraction process comprises the non-dissolution of significant amounts of carbohydrates, polysaccharides, fibers and other

essential compounds present in the biomass. The thermo gravimetric profiles of our biomass before and after extraction mediated by the $[N_{1,1,1,10}]Br$ aqueous solution are identical (Figure S4 from ESI), thus confirming the integrity of the polymeric matrix, and consequently the high selectivity of the IL applied in this process. Focusing the results obtained for the carbohydrates (the most abundant class of compounds in this biomass), no significant changes were detected on the amounts of xylose, glucose and uronic acids present after extraction (Table S6 from ESI).

**Environmental evaluation of the carbon footprint and complete E-factor.** The environmental sustainability of the process developed in this work was evaluated through the carbon footprint and complete E-factor parameters. Data on the amounts of chemicals, water and electricity consumed were obtained during the experiments (Table S1 from ESI). Data on Greenhouse gas (GHG) emission factors from the production of chemicals, water and electricity were taken from Ecoinvent database version 3.5 (Ecoinvent, 2018) (Table S2 from ESI).[36] The results of both metrics are shown in Figure 5 and Table 1. In this analysis, four scenarios were considered. *Scenarios 1* and *2* representing the methods of extraction using aqueous solutions of $[N_{1,1,1,10}]Br$ without and with the cycles of IL and water reuse, respectively; and *Scenarios 3* and *4* for the conventional methods using acetone and ethanol, respectively. Since different extraction yields of carotenoids were obtained in each scenario, the metric results are expressed by 1 μg of extracted carotenoids, allowing thus the direct comparison between scenarios (Table S7 from ESI).

In terms of carbon footprint, Figure 4 and Table S1 from ESI show that ***Scenarios 1*** and ***2*** have lower carbon footprint values than ***Scenarios 3*** and ***4***. The ***Scenario 2*** considers the recovery and reuse of $[N_{1,1,1,10}]Br$ and water, with equivalent results compared to **Scenario 1** (ANOVA $p > 0.05$), carbon footprint at 5.2 g $CO_{2\ eq}.\mu g^{-1}_{carotenoids}$ equivalent to ***Scenario 1*** (4.8 g $CO_{2\ eq}.\mu g^{-1}_{carotenoids}$). This occurs because, despite the

462 reduction of the IL used in ***Scenario 2***, there is also a decrease in the carotenoids
463 extraction yield throughout the three new cycles of extraction. The main contribution to
464 the carbon footprint of ***Scenarios 1*** and ***2*** comes from the electricity consumption, mainly
465 from the evaporation and centrifugation units (53% and 47% of the total carbon footprint
466 for both scenarios, respectively), while the contribution of the IL for both ***Scenarios 1***
467 and ***2*** is practically zero.

468 The ***Scenario 3*** (conventional method using acetone) has the worst environmental
469 performance, with a carbon footprint of 11.6 g $CO_{2\ eq}.\mu g^{-1}_{carotenoids}$. In this scenario, the use
470 of ethyl ether and the electricity consumption represent the major contributions to the
471 total carbon footprint, with 47% and 43% (mostly from centrifugation), respectively. The
472 acetone is only responsible for 3% of the total carbon footprint of this scenario. Finally,
473 ***Scenario 4*** (conventional method using ethanol) has a total carbon footprint of
474 9.3 g $CO_{2\ eq}.\mu g^{-1}_{carotenoids}$. In this scenario, the ethyl ether and the electricity consumption
475 are again the main contributors to the total carbon footprint, with 46% and 42% (mostly
476 from centrifugation), respectively. The ethanol is only responsible for 5% of the total
477 carbon footprint. These results suggest that besides the IL-mediated downstream
478 processes being excellent performances regarding the yield of extraction, the alternative
479 carotenoids polishing performed by water as an anti-solvent are also extremely useful to
480 mitigate the environmental impact comparing with the diethyl and petroleum ether
481 polishing techniques, since at the scenarios 3 and 4 there are an intense impact regarding
482 the use of these conventional solvents.

483 In terms of complete E-factor, Table 1 shows that the recovery and reuse of the IL-
484 based aqueous solutions and water after the polishing steps resulted in a 99% reduction
485 of the complete E-factor in comparison with ***Scenario 1***. In this scenario, the complete E-
486 factor is 0.3 $g_{waste}.\mu g^{-1}_{carotenoids}$, because of the discarded IL and water. By applying the

conventional methods using acetone (***Scenario 3***) and ethanol (***Scenario 4***), 185.1 $g_{waste}.\mu g^{-1}_{carotenoids}$ and 146.1 $g_{waste}.\mu g^{-1}_{carotenoids}$, were respectively generated. Finally, for ***Scenarios 3*** and ***4***, the discarded water contributes to almost 100% of the complete E-factor.

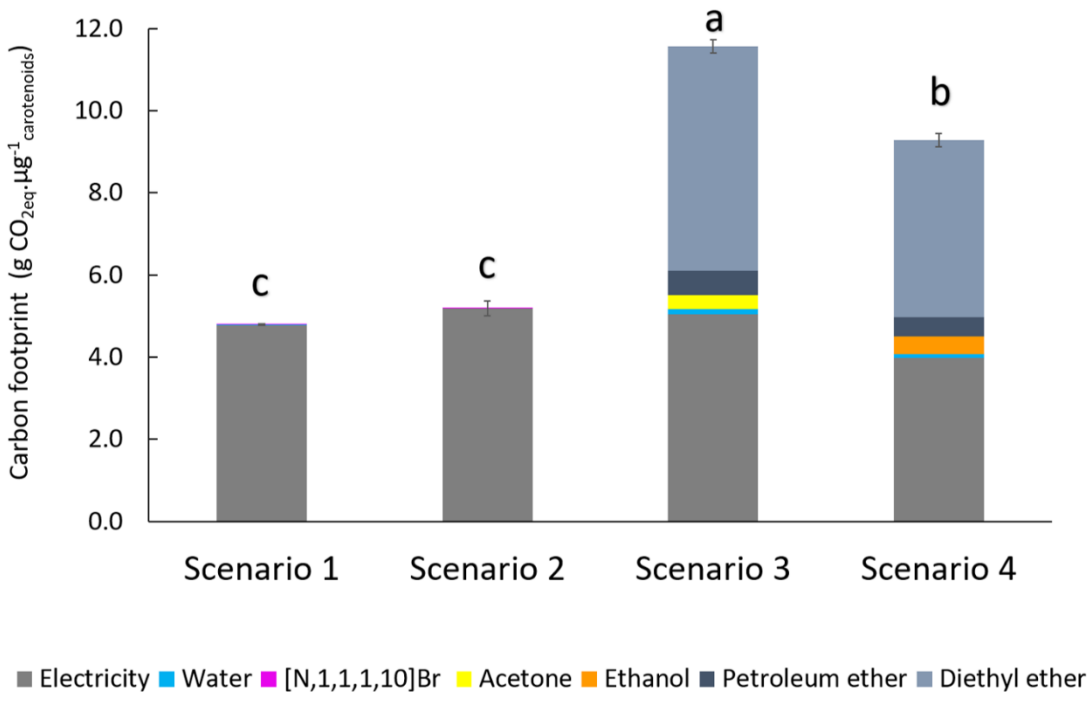



**Figure 5**. The carbon footprint of the four scenarios investigated in this work. Scenarios 1 and 2 for the methods of extraction using aqueous solutions of $[N_{1,1,1,10}]Br$ without and with three cycles of IL and water reuse, respectively; and Scenarios 3 and 4 for the conventional methods using acetone and ethanol, respectively.

**Table 1**. Complete E-factor of the integrated process for obtaining carotenoids in four different scenarios. Scenario 1 – method with $[N_{1,1,1,10}]Br$ without recovery and reuse of the IL-based aqueous solution and water from extraction and polishing stages; Scenario 2 – method with $[N_{1,1,1,10}]Br$ with recovery and reuse of the IL-based aqueous solution and water from extraction and polishing stages; Scenario 3 – conventional method using acetone; Scenario 4 – conventional method using ethanol.

| | Complete E-factor ($g_{waste}.\mu g^{-1}_{carotenoids}$) | | | |
|---|---|---|---|---|
| **Waste generated** | ***Scenario 1*** | ***Scenario 2*** | ***Scenario 3*** | ***Scenario 4*** |
| **$[N_{1,1,1,10}]Br$** | $2.3 \times 10^{-3}$ | 0 | – | – |
| **Acetone** | – | – | 0.1 | – |
| **Ethanol** | – | – | – | 0.1 |
| **Water** | 0.3 | $4.6 \times 10^{-3}$ | 182.9 | 144.4 |
| **Petroleum ether** | – | – | 1.3 | 1.0 |
| **Diethyl ether** | – | – | 0.8 | 0.6 |
| **Total** | 0.3 | $4.6 \times 10^{-3}$ | 185.1 | 146.1 |

**Preparation and characterization of chitosan films.** The development of an alternative food-packaging biomaterial with the incorporation of bioactive compounds into edible films and other biomaterials has become a feature nowadays.[53,54] In addition, the recently the Food and Drug Administration (FDA) recognizes as safe (GRAS status) the incorporation into biodegradable films of compounds derived from fruit pulps.[55] However, most studies focus on the use of phenolic compounds and their role in the biological properties of the films, such as antioxidant, antimicrobial and anti-inflammatory activities.[56–58] Despite the great interest in this field, the incorporation in edible films of an extract rich in carotenoids has not yet been evaluated. In this work, chitosan films were used as a matrix for the incorporation of carotenoids from *B. gasipaes* fruits in three different doses (0.025 %, 0.050 %, and 0.100 % (w/w)), obtained from two types of extraction, the here proposed using the IL as solvent, and the extract obtained using acetone (Figure S5 from ESI).

The mechanical properties of the films were determined in order to evaluate the influence of the carotenoids incorporation, since they could modify the structure of the polymer matrix, by weakening the inter-chain bonds[59] and, consequently, modifying the films mechanical properties.[60] The chitosan-based films without carotenoids (control) had a tensile strength ($\sigma_b$) of 37 MPa, a Young's modulus 1.2 MPa and an elongation at break ($E_b$) of 32%. The effects of incorporating carotenoids in different concentrations on

chitosan-based films are shown in Table 2. The film thickness was evaluated, since it is an important parameter that influences the mechanical properties of the films.[61] The addition of carotenoids, regardless of dose, does not modify the thickness of the films compared to the control group (ANOVA $p > 0.05$), which reflects the equivalent contact area of the materials. All films composed of carotenoids have significant differences in the mechanical properties when compared to control (Table 3 and Figure S5 from ESI). The incorporation of the carotenoids obtained by the IL-based process, at the doses of 0.025 % and 0.050 %, do not change the tensile strength limit of the films (ANOVA $p > 0.05$). On the contrary, the incorporation of carotenoids at the dose of 0.100% obtained by using IL as solvent and carotenoids obtained by acetone extractions (all doses), decreased the tensile strength, meaning the decrease of the films' mechanical resistance. The differences between the mechanical behavior of the films could be justified by the effect of the carotenoids crystallization after the film storage process, which justifies that, for the highest dose of carotenoids extracted by IL (0.100%), the film has a lower tensile strength,[62] contrarily to what happened in the work of Liu and collaborators,[63] where the addition of curcumin to the chitosan-films increased the tensile strength.

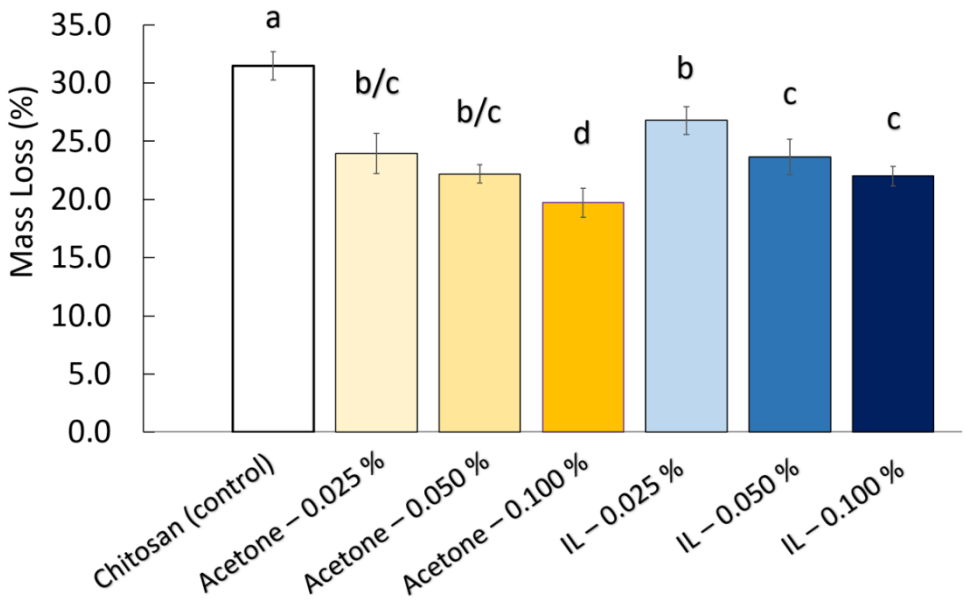
A higher elasticity (elongation at break) of the chitosan-based membranes was obtained by the incorporation of 0.100% of carotenoids obtained by the IL-based process (49 %) when compared with the control films (32 %), whereas the incorporation of 0.100% of carotenoids extracted by acetone have the lowest elasticity (12 %). In addition, all films with carotenoids incorporated showed lower Young's modulus values in comparison with the control, which means their higher ductility. Although all films already have 0.1% of glycerol used as plasticizer, the addition of carotenoids influenced this parameter, which seems to be attributed to the plasticizer action that carotenoids could have on the carbohydrates network.[59]

Comparing the enriched films of carotenoids obtained with IL and acetone, it seems that the viscoelasticity properties are significantly different (Table 2). For example, at 0.100 % dose, the films enriched with carotenoids extracted by IL resemble elastomers (E of 49 %), while the films enriched with carotenoids extracted by acetone have E of 12%, besides low tensile strength. Despite the wide range of applications of natural biopolymers, the elastomer-like ones possess a robust interface,[64] since they can be molded by a wide range of processes, due to their softness, flexibility, and resilience.[65] The surface wettability of the chitosan-based films incorporating the carotenoids was evaluated by contact angle analysis using ultrapure water. The contact angle of all films with different carotenoids doses are listed in Table 3. The films with carotenoids incorporated revealed a slightly lower contact angle value in both film phases. These results are somehow contrary to what was expected, a phenomenon that could be explained by the amorphous and crystalline surfaces obtained after the incorporation of carotenoids in the chitosan network bounds;[66] or by the spatial condition of the carbon skeleton between the chitosan bounds, as previously reported for the addition of curcumin also in chitosan-based membranes;[63] or even by the potential contamination of the extracts derived from acetone extraction (acetone is not a selective solvent).

The films solubility was also determined after 7 days immersed in a mixture of 50:50 (v/v) of water:ethanol under continuous stirring. Figure 6 shows the weight loss percentage for the films with carotenoids extracted with acetone and $[N_{1,1,1,10}]Br$ as solvents, as well as one film prepared only with chitosan (without carotenoids incorporated) used as control. The control film presented the higher weight loss percentage (32%), comparing with the weight loss determined for films prepared with carotenoids obtained with acetone and IL as solvents (20-27%). The films prepared with the carotenoids derived from the use of acetone as solvent showed a slightly lower

572 solubility, especially for the film with 0.100% of carotenoids extract (*T*-test: $p < 0.05$),
573 when compared with the film incorporating the carotenoids extracted by the IL. Summing
574 up, the carotenoids incorporation resulted in a decrease in the films solubility, due to the
575 hydrophobicity of carotenoids incorporated in the chitosan matrix, however with no loss
576 of the IL remaining in the carotenoids extract (around 0.17%) to the water, which was
577 proved by the absence of bromide anions determined by ionic chromatography, thus
578 demonstrating the development of a safe biomaterial.

579 The antioxidant activity of the films was also tested for three different periods after
580 their production, namely 1, 5 and 20 days (Figure S6 from ESI). Briefly, and as expected,
581 the films with carotenoids showed higher antioxidant activity when compared with the
582 control films. It is observed that the maximum dose of carotenoids incorporated in films
583 (0.100%) have the highest antioxidant activity, for both strategies of extraction (IL and
584 acetone). In all doses (0.025, 0.050 and 0.100 %) and for all times tested (15, 30, 60 and
585 120 min) the films enriched with carotenoids obtained by using IL as solvent have the
586 highest antioxidant activity data, which was maintained during the 20 days of evaluation.
587 This can be justified by the highest selectivity of the IL carrying the extraction of
588 carotenoids, when compared with the films incorporating the carotenoids extracted by
589 acetone.[37,38]




590

591 **Figure 6**. Weight loss percentage determined for the chitosan-based films solubility test,
592 after 7 days in a water:ethanol mixture.

593

594 **Table 2**. Mechanical properties of films with and without carotenoids incorporated.
595 Different letters in the same column indicate significant differences (Bonferroni pos-hoc
596 test, $p < 0.05$)

| Films | Young's modulus (*E*) | Tensile strength (MPa) | Elongation at break (%) |
|---|---|---|---|
| **Control** | $1.2 \pm 0.2^{a}$ | $37 \pm 6^{a}$ | $32 \pm 4^{b/c}$ |
| **IL 0.025 %** | $0.6 \pm 0.2^{b}$ | $34 \pm 5^{a}$ | $35 \pm 4^{b}$ |
| **IL 0.050 %** | $0.4 \pm 0.1^{b}$ | $35 \pm 8^{a}$ | $38 \pm 5^{b}$ |
| **IL 0.100 %** | $0.05 \pm 0.02^{c}$ | $18 \pm 1^{b/c}$ | $49 \pm 6^{a}$ |
| **Acetone 0.025 %** | $0.4 \pm 0.1^{b}$ | $22 \pm 3^{b}$ | $29 \pm 6^{c}$ |
| **Acetone 0.050 %** | $0.5 \pm 0.1^{b}$ | $19 \pm 7^{b}$ | $31 \pm 8^{c}$ |
| **Acetone 0.100 %** | $0.53 \pm 0.08^{b}$ | $13 \pm 2^{c}$ | $12 \pm 3^{d}$ |

597

598 **Table 3.** Contact angle (°) measures from chitosan-based films in both bottom and top
599 surfaces. Different letters in the same column indicate significant differences (Bonferroni
600 pos-hoc test, $p < 0.05$).

601

| Films | Bottom phase | Top phase |
|---|---|---|
| **Control** | $108 \pm 8^{a}$ | $100 \pm 7^{a}$ |
| **IL 0.025 %** | $92 \pm 4^{b}$ | $90 \pm 5^{b}$ |
| **IL 0.050 %** | $85 \pm 6^{b/c}$ | $80 \pm 4^{b}$ |
| **IL 0.100 %** | $87 \pm 3^{b}$ | $76 \pm 6^{b}$ |
| **Acetone 0.025 %** | $93 \pm 7^{b}$ | $77 \pm 9^{b}$ |
| **Acetone 0.050 %** | $91 \pm 3^{b}$ | $72 \pm 6^{b/c}$ |
| **Acetone 0.100 %** | $72 \pm 8^{c}$ | $69 \pm 6^{c}$ |

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608 ## Conclusions

609 An extraction process of all-*trans*-β-carotene, all-*trans*-lycopene and all-*trans*-γ-
610 carotene from the fruit biomass of the Amazonian tree *Bactris gasipaes* was successfully
611 developed in this work using an ionic liquid-based extraction. $[N_{1,1,1,10}]Br$ in water was
612 selected as the most efficient solvent to maximize the selective extraction of carotenoids
613 (yield of extraction of $88.7 \pm 0.9$ $\mu g_{carotenoids}.g_{dried\ biomass}^{-1}$), for the optimum conditions of
614 8.2 minutes, 140 mM of IL and $R_{(S/L)}$ of 0.15. Besides the highest yield of extraction, the
615 use of aqueous solutions of $[N_{1,1,1,10}]Br$ allowed the development of an integrated process
616 in which the isolation of the carotenoids and recyclability of the solvents, IL and water,
617 were successfully and easily achieved by using water as an anti-solvent. In addition, the
618 reuse of the IL for a total of four cycles of extraction was optimized, which helped to
619 significantly decrease the environmental impact of the IL-based process, a result
620 demonstrated by the carbon footprint and complete E-factor assessment. Proved the
621 efficiency and low environmental impact of the process, and after isolation, the
622 carotenoids were applied on the development and optimization of a task-specific

chitosan-based film applicable in food packaging, with very advantageous results obtained for tensile strength, Young's modulus, elongation at break, elasticity, film thickness, wettability, solubility in water and, in particular for antioxidant activity, which was maintained in the highest levels during 20 days of experiment.

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## Conflicts of interest

There are no conflicts to declare.

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